

1. (Amended) A method for identification of biologically active ribonucleic acids or peptides or [their]cellular ligands to the biologically active nucleic acids or peptides, which comprises the steps of

- (a) producing a pool of appropriate vectors each containing a DNA sequence to be examined,
- (b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids and peptides encoded by DNA sequences to be examined,
- (c) screening said transduced cells to see whether some of them have altered a preselected phenotypic trait, said screening being one which does not require knowledge of 1) chains of mechanisms in the cell, 2) enzymes in the cell, 3) signalling pathways in the cell, or 4) receptors in the cell which generate the preselected phenotypic trait, and
- (d) selecting and cloning cells which have altered the preselected phenotypic trait,

wherein the pool of appropriate vectors in step (a) contains totally or partly random DNA sequences selected from the group consisting of:

- i) synthetic totally random DNA sequences;
- ii) synthetic random DNA sequences wherein stop codons are absent;
- iii) synthetic DNA sequences which encode random amino acid sequences with an even distribution of amino acids;
- iv) synthetic DNA sequences defined in (i), (ii), or (iii) separated by codons which enable specific post-translational

modifications of all expressed peptides or which encode anchor residues;

- v) synthetic DNA sequences defined in (i), (ii), (iii), or (iv) coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides; and
- vi) synthetic DNA sequences defined in (i), (ii), (iii), (iv), or (v) coupled to or inserted into the coding sequence of a protein;

and wherein

- (e) the vector DNA in the phenotypically altered cells is isolated and sequenced, and the sequences of the ribonucleic acids or peptides effecting alteration of the preselected phenotypic trait are deduced from the sequenced vector DNA;

and/or

- (f) the ribonucleic acids or peptides effecting alteration of the preselected phenotypic trait are used directly for isolation and identification of a ligand molecule to said ribonucleic acids or peptides.

26. (Amended) The method according to claim 1, wherein the alteration of the preselected ^{cellular function} phenotypic trait is up-regulation or down-regulation of expression of a cell surface[expression of a] protein.

32. (Amended) A method for identification of biologically active ribonucleic acids or peptides or [their] cellular ligands to the biologically active ribonucleic acids or peptides, which comprises the steps of

- (a) producing a pool of appropriate vectors each containing a DNA sequence to be examined,
- (b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids and peptides encoded by DNA sequences to be examined,
- (c) screening said transduced cells to see whether some of them have altered a preselected phenotypic trait, wherein the screening method is different from detection of interaction between [capture of]the expressed ribonucleic acid(s) or peptide(s) [with a ligand] and a preselected enzyme or receptor, and
- (d) selecting and cloning cells which have altered the preselected phenotypic trait,

wherein the pool of appropriate vectors in step (a) contains totally or partly random DNA sequences selected from the group consisting of:

- i) synthetic totally random DNA sequences;
- ii) synthetic random DNA sequences wherein stop codons are absent;
- iii) synthetic DNA sequences which encode random amino acid sequences with an even distribution of amino acids;
- iv) synthetic DNA sequences defined in (i), (ii), or (iii) separated by codons which enable specific post-translational modifications of all expressed peptides or which encode anchor residues;
- v) synthetic DNA sequences defined in (i), (ii), (iii), or (iv) coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides; and

vi) synthetic DNA sequences defined in (i), (ii), (iii), (iv), or (v) coupled to or inserted into the coding sequence of a protein;

and wherein

(e) the vector DNA in the phenotypically altered cells is isolated and sequenced, and the sequences of the ribonucleic acids or peptides effecting alteration of the preselected phenotypic trait are deduced from the sequenced vector DNA;

and/or

(f) the ribonucleic acids or peptides effecting alteration of the preselected phenotypic trait are used directly for isolation and identification of a ligand molecule to said ribonucleic acids or peptides.

33. (Amended) A method for identification of biologically active ribonucleic acids or peptides or [their] cellular ligands to the biologically active ribonucleic acids or peptides, which comprises the steps of

- (a) producing of a pool of appropriate vectors each containing a DNA sequence to be examined,
- (b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids and peptides encoded by DNA sequences to be examined,
- (c) screening said transduced cells to see whether some of them have altered a preselected phenotypic trait, where alteration of the preselected phenotypic trait in a cell [is ascribable

to the expressed ribonucleic acid(s) or peptide(s) affecting biological functions of the cell which have influence on the preselected phenotypic trait] indicates that ribonucleic acid(s) or peptide(s) encoded by the vector DNA affect(s) biological functions in the cell thereby effecting the alteration of the preselected phenotypic trait, and

- (d) selecting and cloning cells which have altered the preselected phenotypic trait,

wherein the pool of appropriate vectors in step (a) contains totally or partly random DNA sequences selected from the group consisting of:

- i) synthetic totally random DNA sequences;
- ii) synthetic random DNA sequences wherein stop codons are absent;
- iii) synthetic DNA sequences which encode random amino acid sequences with an even distribution of amino acids;
- iv) synthetic DNA sequences defined in (i), (ii), or (iii) separated by codons which enable specific post-translational modifications of all expressed peptides or which encode anchor residues;
- v) synthetic DNA sequences defined in (i), (ii), (iii), or (iv) coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides; and
- vi) synthetic DNA sequences defined in (i), (ii), (iii), (iv), or (v) coupled to or inserted into the coding sequence of a protein;

and wherein

- (e) the vector DNA in the phenotypically altered cells is isolated and sequenced, and the sequences of the ribonucleic acids or

Serial No.: 08/973,021

Atty. Docket No.: 303/P61724US0

peptides effecting alteration of the preselected phenotypic trait are deduced from the sequenced vector DNA;
and/or

- (f) the ribonucleic acids or peptides effecting alteration of the preselected phenotypic trait are used directly for isolation and identification of a ligand molecule to said ribonucleic acids or peptides.

Cancel claims 34-36 without prejudice or disclaimer.

41. (Amended) The method according to claim 1, wherein the ribonucleic acids or peptides effecting alteration of the preselected ~~phenotypic trait~~ ^{cellular target} are used directly for isolation of a [ligand molecule] cellular target protein of the identical cells to said ribonucleic acids or peptides.

42. (Amended) A method for identification of biologically active ribonucleic acids or peptides or [their] cellular ligands to the biologically active ribonucleic acids or peptides, which comprises the steps of

- (a) producing a pool of appropriate vectors each containing a DNA sequence to be examined,
(b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids and peptides encoded by DNA sequences to be examined,

- (c) screening said transduced cells to see whether some of them have [altered] up-regulated or down-regulated a preselected [phenotypic trait, said phenotypic trait being an observable characteristic of the identical eukaryotic cells prior to and after transduction] biological effect, and
- (d) selecting and cloning cells which have [altered] up-regulated or down-regulated the preselected [phenotypic trait] biological effect,

wherein the pool of appropriate vectors in step (a) contains totally or partly random DNA sequences selected from the group consisting of:

- i) synthetic totally random DNA sequences;
- ii) synthetic random DNA sequences wherein stop codons are absent;
- iii) synthetic DNA sequences which encode random amino acid sequences with an even distribution of amino acids;
- iv) synthetic DNA sequences defined in (i), (ii), or (iii) separated by codons which enable specific post-translational modifications of all expressed peptides or which encode anchor residues;
- v) synthetic DNA sequences defined in (i), (ii), (iii), or (iv) coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides; and
- vi) synthetic DNA sequences defined in (i), (ii), (iii), (iv), or (v) coupled to or inserted into the coding sequence of a protein;

and wherein

- (e) the vector DNA in the [phenotypically altered] selected and cloned cells is isolated and sequenced, and the sequences of

the ribonucleic acids or peptides effecting [alteration of the preselected phenotypic trait] up-regulation or down-regulation of the preselected biological effect are deduced from the sequenced vector DNA;

and/or

- (f) the ribonucleic acids or peptides effecting[alteration of the preselected phenotypic trait] up-regulation or down-regulation of the preselected biological effect are used directly for isolation and identification of a ligand molecule to said ribonucleic acids or peptides.

Add the following new claims:

--43. A method for identification of cellular target proteins to biologically active nucleic acids or peptides, which comprises the steps of

- (a) producing a pool of appropriate vectors each containing a DNA sequence to be examined,
- (b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids and peptides encoded by DNA sequences to be examined,
- (c) screening said transduced cells to see whether some of them have altered a preselected phenotypic trait, and
- (d) selecting and cloning cells which have altered the preselected phenotypic trait,

wherein the pool of appropriate vectors in step (a) contains totally or partly random DNA sequences selected from the group consisting of:

- i) synthetic totally random DNA sequences;
- ii) synthetic random DNA sequences wherein stop codons are absent;
- iii) synthetic DNA sequences which encode random amino acid sequences with an even distribution of amino acids;
- iv) synthetic DNA sequences defined in (i), (ii), or (iii) separated by codons which enable specific post-translational modifications of all expressed peptides or which encode anchor residues;
- v) synthetic DNA sequences defined in (i), (ii), (iii), or (iv) coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides; and
- vi) synthetic DNA sequences defined in (i), (ii), (iii), (iv), or (v) coupled to or inserted into the coding sequence of a protein;

and wherein

- (e) the ribonucleic acids or peptides encoded by the DNA to be examined and present in the cells which have altered the preselected phenotypic trait are used directly for isolation and identification of cellular target proteins to said ribonucleic acids or peptides.

44. The method according to claim 43, wherein alteration of the preselected phenotypic trait in step (c) indicates that ribonucleic acid(s) or peptide(s) encoded by the vector DNA affect(s) biological functions in the cell thereby effecting the alteration of the preselected phenotypic trait

45. The method according to claim 43, wherein the alteration of the preselected phenotypic trait is up-regulation or down-regulation of a biological effect of the cell.

46. The method according to claim 43, wherein the screening is selected so as not to require knowledge of 1) chains of mechanisms in the cell, 2) enzymes in the cell, 3) signalling pathways in the cell, or 4) receptors in the cell which regulate the preselected phenotypic trait.

47. The method according to claim 45, wherein the biological effect is expression of a cell-surface protein.

48. The method according to claim 1, wherein the eukaryotic cells are mammalian.

49. The method according to claim 32, wherein the eukaryotic cells are mammalian.

50. The method according to claim 33, wherein the eukaryotic cells are mammalian.

Serial No.: 08/973,021

Atty. Docket No.: 303/P61724US0

51. The method according to claim 42, wherein the eukaryotic cells are mammalian.

52. The method according to claim 43, wherein the eukaryotic cells are mammalian.

53. The method according to claim 1, wherein the identical eukaryotic cells are cells of a cell clone or a cell line.

54. The method according to claim 32, wherein the identical eukaryotic cells are cells of a cell clone or a cell line.

55. The method according to claim 33, wherein the identical eukaryotic cells are cells of a cell clone or a cell line.

56. The method according to claim 42, wherein the identical eukaryotic cells are cells of a cell clone or a cell line.

57. The method according to claim 43, wherein the identical eukaryotic cells are cells of a cell clone or a cell line.

58. In a drug development method wherein a target molecule serves to identify candidate drugs, the improvement comprising that a cellular target protein which has been identified according to claim 43 is the target molecule.--